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Phytophthora morindae, a new species causing black flag disease on noni (*Morinda citrifolia* L) in Hawaii

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Abstract: A homothallic, papillate *Phytophthora* species causing foliar and fruit blight of noni (*Morinda citrifolia* var. *citrifolia*) in Hawaii was identified. The asexual phase of this species is characterized by the production of umbellate sporangiophores and papillate sporangia that are ellipsoid and obpyriform with conspicuously tapered bases and possess caducous, medium to long pedicels. The sexual phase is characterized by the production of oogonia with tapered bases, small amphigynous antheridia and thick-walled, plerotic oospores. The morphology of the taxon does not match any of the valid 95 *Phytophthora* species described to date. Phylogenetic analysis based on sequences of the internal transcribed spacer rDNA region (ITS) and the translation elongation factor 1 α (EF-1α) of this taxon and those from other *Phytophthora* species from GenBank and the *Phytophthora* database indicates that the new taxon is most closely related to species in ITS clade 10, including *P. kernoviae*, *P. boehmeriae* and the recently described *P. gallica*. The most closely related species is *P. kernoviae*, an invasive plant pathogen causing bleeding stem lesions on forest trees (beech, *Fagus sylvatica*) and foliar necrosis of ornamentals (rhododendron, pieris and magnolia) in the UK, and isolated in New Zealand from necrotic cherimoya shoots and fruits and soil. Although the morphological characters of the sexual phase of *P. morindae* and *P. kernoviae* are similar, the umbellate sporangiophores produced by the new taxon marks the main morphological distinction. In this paper we describe the morphological characteristics, the phylogenetic relationships and pathogenicity characteristics that support the description of this taxon as a new species with the proposed name *Phytophthora morindae* sp. nov.

Key words: EF-1A, ITS, morphology, phylogenetics, *Phytophthora morindae* sp. nov., taxonomy

INTRODUCTION

A severe foliar blight and fruit rot disease of noni (Indian mulberry, *Morinda citrifolia* L. var. *citrifolia*), named black flag disease by the first author, was discovered in the Puna District near Opihikao on the island of Hawaii in 1999 (FIGS. 1–5). All aboveground, nonwoody organs of *M. citrifolia* are susceptible to infection, including vertical and lateral succulent stems, petioles, bracts, stipules, leaves, syncarps (fruits) and flowers. *Morinda citrifolia* roots and the tissues of woody trunks and stems show no symptoms and are not infected by the pathogen. The disease spread throughout naturalized populations of *M. citrifolia* growing in the native coastline forests and to noni orchards in the district. Noni originally was brought to the area by seafaring Polynesians who colonized the island and thereafter spread naturally within forests and to recent lava flows and to disturbed areas. Epidemics of the disease recur periodically after periods of extended and heavy rainfall. A requirement for the disease appears to be the presence of a significant amount of free water on the surface of foliage. The disease is caused by a new pathogen in genus *Phytophthora*.

Severely diseased plants have characteristic black flags (i.e. diseased leaves), which describes the appearance of the blackened, wilted, withered or necrotic leaves that hang conspicuously for lengthy periods from blackened petioles and stems. In early stages of infection the leaves, petioles and stems may have blackened streaks or stripes along the veins that correspond to rapid mycelial invasion of leaf vasculature and adjacent tissues. As the disease progresses entire stems and petioles may collapse after being girdled and rotted by dark lesions. On the other hand leaves may have light brown to black spots ranging from several millimeters to several centimeters diameter. Spots tend to coalesce rapidly within several days, blighting leaves. Fruit symptoms, commonly starting at the stem end, consist of a progressive, hydrotic soft rot, which is chocolate brown or dark brown to black. Fruit infection often occurs through the pedicel, where the base of the fruit is attached to

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FIGS. 1–4. Symptoms of the black flag disease caused by *Phytophthora morindae*. 1. Fruit symptoms in an early stage (demonstrates that green fruits are attacked before they are ripe). 2. All symptoms types: black flags, veinal necrosis, fruit rot, fruit mummification, leaf spot, leaf blight and stem blight. 3. Effect of the disease at a local farm (widespread defoliation and fruit drop). 4. Foliar blight can cause normal green leaves to turn black in approximately 1 wk after infection. Location: Leilani Estates, island of Hawaii.

the stem. Advanced fruit infections may result in dry, shriveled fruit mummies that may have a fuzzy or silvery surface due to colonization by saprophytic fungi; fruit mummies may remain attached to necrotic stems. Rots of stems and petioles can progress apically and basipetally from the point of infection, which is usually the fruit peduncle or stem stipules.

On severely diseased plants during extraordinarily wet periods or after many days of continuous rain almost all photosynthetic, succulent plant tissues can die. Significant destruction of plant tissues can occur within 7–10 d after inoculation. Defoliation and fruit drop can be extensive. Plant death can occur, although older plants with denuded woody stems can sprout new growth during drier weather when the disease is not active. The dried, dead leaves and stems may hang from trees for weeks or months, until the dead foliage decomposes and new stem and foliage regrowth occurs.

Phytophthora, an Oomycete in kingdom Straminipila (Dick 2001), is one of the most important genera of plant pathogens. The genus contains about 95 valid species, some historically devastating, including *P.*

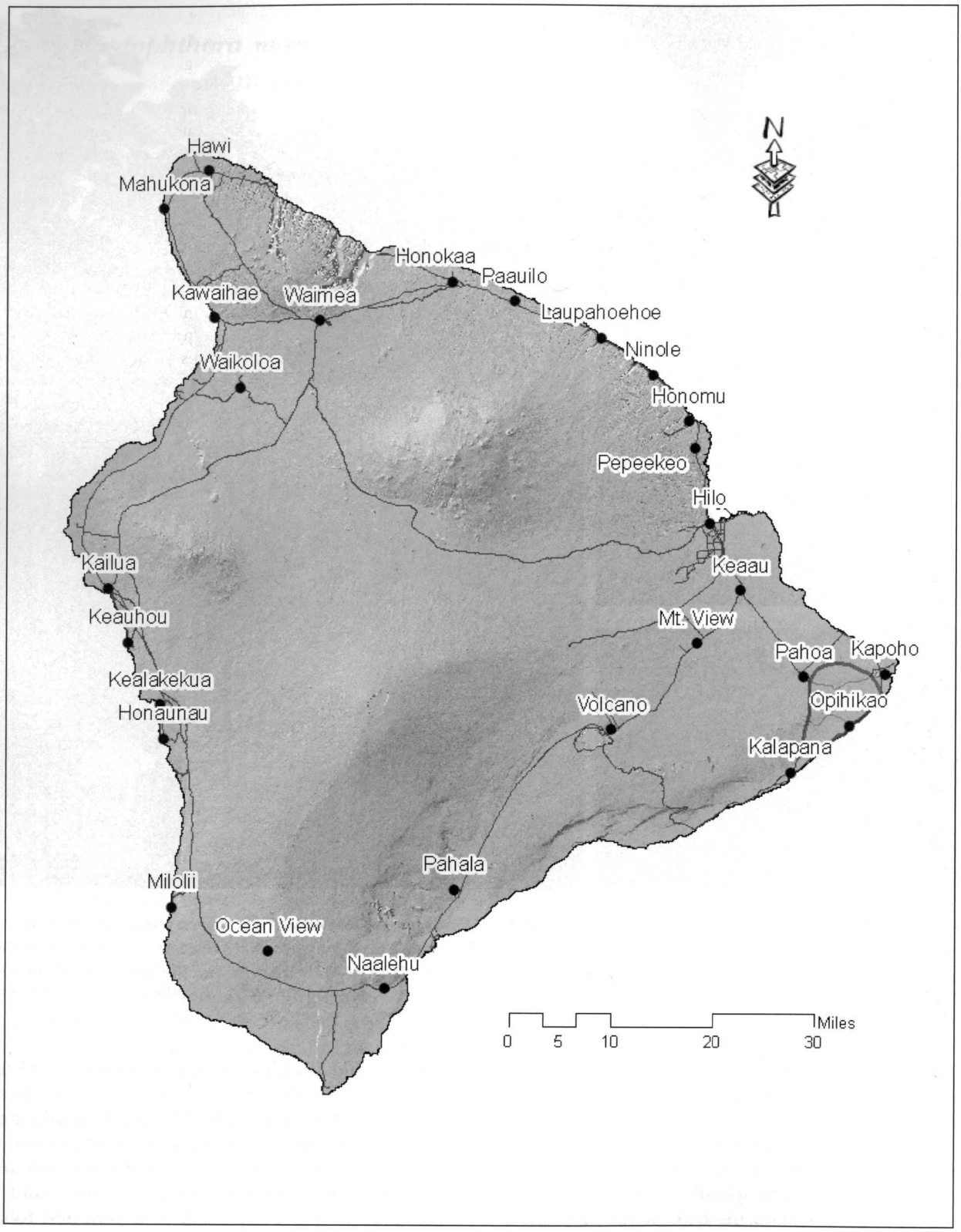


FIG. 5. Area surrounding Opihikao, in which *Phytophthora morindae* is causing black flag disease of *Morinda citrifolia* on the island of Hawaii.

cinnamomi Rands, *P. infestans* (Mont.) de Bary and *P. ramorum* Werres, de Cock & Man in 't Veld. Fifty-nine morphological species are presented in the *Phytophthora* treatise of Erwin and Ribeiro (1996), and 34 new species were reported recently with integrated morphological and molecular supporting data. Nineteen of the recently described species are cited in Abad et al 2008, and the following are the most recently described: *P. alticola* Maseko, Coutinho & M.J. Wingf. (2007), *P. austrocedrae* Gresl. & Hansen (2007), *P. bisheria* Abad ZG, Abad J.A. & Louws (2008), *P. frigida* Maseko, Coutinho & MJ Wingf. (2007), *P. gallica* T Jung & J Nechwatal (2008), *P. irrigata* C Hong & M Gallegly (2008), *P. multivora* PM Scott & T Jung (2009), *P. parsiana* Mostowfizadeh, Cooke & Banihashemi (2008), *P. pinifolia* A.J. Durán, Gryzenh. & M.J. Wingf. (2008), *P. plurivora* T. Jung and T.I. Burgess (2009), *P. quercetorum* Balci & Balci (2008), *P. rosacearum* EM Hansen & Wilcox (2009), *P. sansomeana* EM Hansen & Reeser (2009) and *P. siskiyouensis* Reeser & EM Hansen (2007) (Abad et al 2008, Balci et al 2008, Duran et al 2008, Greslebin et al 2007, Hansen et al 2009, Hong et al 2008, Jung and Nechwatal 2008, Jung and Burgess 2009, Maseko et al 2007, Mostowfizadeh-Ghalamfarsa et al 2008, Reeser et al 2007, Scott et al 2009).

Validation of new *Phytophthora* taxa in recent years has been supported with powerful phenologies generated by sequence analysis of the internal transcribed spacer rDNA region (ITS), translation elongation factor 1 alpha (EF-1α), β tubulin, and mitochondrial encoded citochrome oxidase Cox I and II (Cooke et al 2000, Kroon et al 2004, Martin and Tooley 2003). A multilocus phylogeny for 82 *Phytophthora* species using seven molecular markers has been published showing the presence of 10 well supported clades for the genus (Blair et al 2008).

Sequences from the internal transcribed spacer rDNA (ITS), translation elongation factor 1 alpha (EF-1α), beta tubulin (β Tub), 60S ribosomal protein L10, enolase (ENL), heat shock protein 90 (HSP90), large subunit rRNA (28S LSU), mitochondrial Cox I and Cox II, and triosephosphate isomerase/glyceraldehyde-3-phosphate dehydrogenase (tigA) for about 90 of the reported *Phytophthora* species are available at the National Center for Biotechnology Information (NCBI). In this study we integrated morphological and molecular data to describe this taxon as a new *Phytophthora* species.

MATERIALS AND METHODS

Isolation and morphological characterization.—Symptomatic tissues of *M. citrifolia* var. *citrifolia* with apparent surface sporulation of a fungus or fungus-like organism were collected from an orchard of *M. citrifolia* var. *citrifolia* in Opihikao on the island of Hawaii and observed microscopically at 100×. Mycelium, sporangia, oogonia and oospores typical for *Phytophthora* species were detected. Pieces of leaves, petioles, fruits and stems of symptomatic plants were thoroughly washed with tap water to remove dust, soil particles and plant debris. Small sections from the margins of lesions at the interface with asymptomatic tissue were selected and surface sterilized 1 min with a solution of 5% bleach (sodium hypochlorite, Clorox). Sections were rinsed in sterile distilled water 1 min and blotted dry on sterile absorbent paper. Treated tissue pieces were placed in Petri dishes on water agar containing 15 gm Bacto agar. Single hyphal tips of emerging colonies were transferred to cornmeal agar (CMA) and incubated 14 d at room temperature (25 C). The colonies on CMA were identified tentatively as a member of genus *Phytophthora* on the basis of sporangial and vegetative characteristics. Isolates on CMA were sent to Drs M. Aragaki and W.-H. Ko at the University of Hawaii at Manoa and D.B. Rayford at CABI Biosciences in UK. All mycologists confirmed that the organism shared some morphological features with *P. botryosa* Chee, especially the sporangia (A. Aragaki, W. Ko, D.B. Rayford pers comm).

Isolates were submitted to the USDA-APHIS-Molecular Diagnostics Laboratory in Beltsville, Maryland, for the morphological and molecular characterization of the species. Ten isolates were grown on CMA P_{10}ARP selective medium containing pimaricin, ampicillin, rifampicin and pentachloronitrobenzene without hymexazol (Kanwischer and Mitchell 1978). Isolates were transferred into potato dextrose agar 30 (PDA, Difco 30 gr/lt), baby carrot agar (B-CA), and baby lima bean agar B-LBA. Both media were prepared with 50 g substrate/500 mL water, autoclaved 5 min and filtered; media totaled 1 L with the addition of 17 g agar. Sporangia and oospores were produced abundantly in both B-CA and B-LBA. B-LBA was used for the morphological characterization of the isolates. Measurements of 50 structures of the asexual and sexual stages were performed for each isolate after 15 d growth in darkness for the characterization of the species.

Isolates were evaluated morphologically to species with keys for identification of *Phytophthora* species (Waterhouse 1970, Stamps et al 1990, Erwin and Ribeiro 1996, Gallegly and Hong 2008) and a morphological/phylogenetic key for Oomycetes, *Phytophthora* (Abad ZG unpubl). Colony morphology and analysis of the minimum, optimum and maximum cardinal growth temperatures were performed in B-LBA, B-CA and PDA-30. Cultures of the organism were maintained on slants of B-CA and in sterile water cultures (glass tubes with 15 mL distilled sterilized water) during this study.

Molecular characterization.—Five isolates of the putative new *Phytophthora* species were grown 7 d in potato dextrose broth (Difco, USA) and DNA was extracted with the PUREGENE DNA isolation kit (Gentra Systems Inc. Minneapolis, Minnesota) according to manufacturer's protocols. Concentration and quality of total DNA were estimated by electrophoresis. Universal oligonucleotide

primers ITS5 and ITS4 (White et al 1990) were used to amplify the ITS rDNA region. Oligonucleotide primers ELONGF1 and ELONGR1 were used to amplify the translation elongation factor 1 alpha (EF-1α) (Kroon et al 2004). PCR amplifications were performed following published protocols (Cooke and Duncan 1997, Kroon et al 2004). Amplicons were purified with the QIAGEN kit according to manufacturer's protocol (QIAGEN Inc., Chatsworth, California). Sequences of the putative new *Phytophthora* species and the closest species in clade 10 and one representative from other clades were selected for final phylogenetic analysis (FIGS. 46–47). A sequence from *Pythium vexans* was used as outgroup. Phylogenetic analysis with the group of selected sequences was carried out in TOPALi (Milne et al 2004) with the Felsenstein-84 nucleotide substitution plus gamma rates heterogeneity model to estimate the pair-wise distances. The tree was estimated with neighbor joining (NJ). Bootstrap support values were derived from 1000 replicates for the NJ, and the tree was exported via TREEVIEW (Page 1996).

Pathogenicity tests.—To prove pathogenicity and fulfill Koch's postulates a series of inoculation tests were conducted with four isolates of *P. morindae* 2000–2005. Forms of inocula and conditions for post-inoculation incubation were evaluated, including inocula consisting of sporangia and plugs of fungal colonies excised from 1–2 wk old colonies on WA and CMA. To obtain plants for inoculation seeds of the large-fruit Hawaiian type of *M. citrifolia* var. *citrifolia* were collected from ripe fruits in the region, scarified and germinated as described (Nelson 2005) and grown in pots of various sizes containing Sunshine Mix 2 (peat and perlite). Plants used in inoculation trials were approximately 1–6 mo old. Three to ten noni plants were inoculated in the individual pathogenicity trials. In each trial an equal number of noninoculated plants (i.e. plants just receiving foliar sprays or applications of distilled water without pathogen propagules) were included as controls. Plants were observed for symptom development daily after inoculation and monitored 2–3 wk.

To obtain sporangia for inoculation isolates were grown 1–2 wk on CMA. Plates were flooded with sterile distilled water and gently shaken or scraped to dislodge sporangia and mycelial fragments. Aqueous suspensions containing approximately 10^3–10^4 sporangia per mL were applied by sterile pipettes in 3–5 mL aliquots to tissues of *M. citrifolia*. Some trials consisted of placing noni plants with inoculated foliage in either clear or opaque (black) plastic bags up to 7 d after inoculation.

TAXONOMY

Phytophthora morindae Z.G. Abad & S.C. Nelson, sp. nov. FIGS. 5–45

MycoBank MB 513008

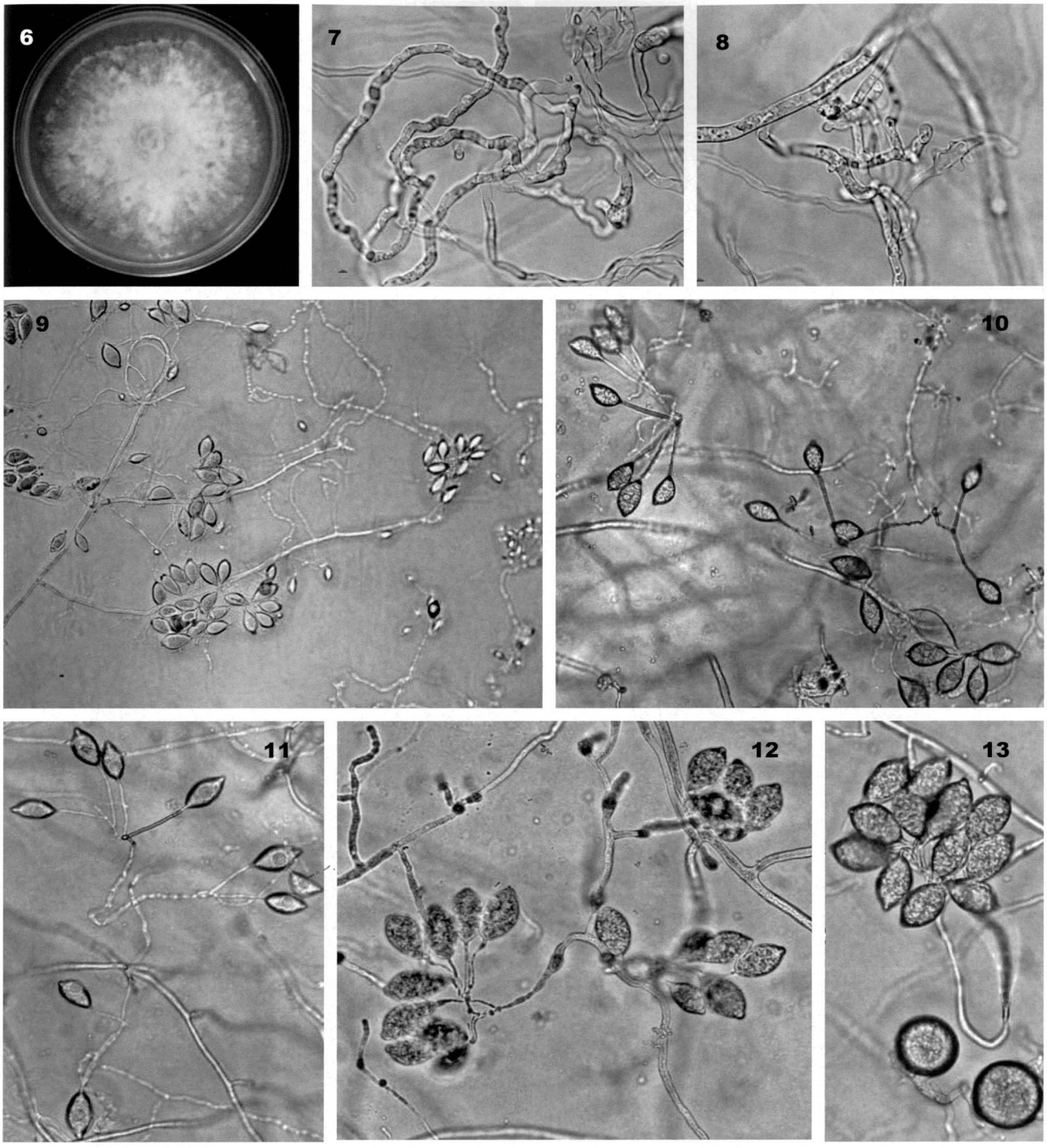
Etymology. morindae refers to the host *Morinda citrifolia* L.

Phytophthora morindae coloniae in agaro carotae adppresae, mycelium aerium sparsum; hyphae primariae 3–8 μm latae. Chlamydosporae ignotae. Sporangiophora umbella forma, sporangia in mediis solidis papillata, ellipsoidea, pyriforme basi attenuata 30–54 (medio 42) μm longa, 19.2–24 (medio 21.8) μm latae; decidua cum medio, longa pedicellulibus. Homothallica, oogonia saepe cum base attenuata, diam 21.6–39.6 (medio 30.4) μm, antheridia amphigynea 7.2–14.4 × 6–12 (medio 11.2 × 8.5) μm, oosporae pleroticae 21–38.9 (medio 30.3) μm diam, parietibus 2.4–3.6 (medio 3.2) μm crassis. Regiones rDNA ITS et EF-1α cum unica sequential (GenBank FJ469147, FJ469148).

Phytophthora morindae Z.G. Abad & S.C. Nelson, sp. nov.

Phytophthora morindae colonies on PDA developed moderate aerial mycelia and a fine chrysanthemum pattern at the border of the colony (FIG. 6), colonies on baby carrot agar with aerial mycelia sparse. Mycelium branched and unbranched with curly hyphae frequently observed in B-LBA (FIGS. 7, 8), main hyphae 3–8 μm wide. Minimum for growth on B-LBA is 10 C, optimum 20 C, maximum 27 C. Colonies on B-LBA slow growing, growth 2.9 mm/d^{-1} at 20 C. Chlamydospores and hyphal swellings were absent in water or culture media. Sporangia were produced abundantly in solid media, including B-CA, B-LBA and water cultures, and formed in umbellate sporangiophores (FIGS. 9–13). Sporangia had conspicuous papillae, usually one but were occasionally bipapillate, were ellipsoid, ellipsoid with tapered base, obpyriform, limoniform or asymmetrical (bilaterally symmetrical or mouse shape with one rounded and one flatter side) (FIGS. 14–29) 30–54 (av 42) μm long, 19.2–24 (av 21.8) μm wide, and were caducous with variable length of pedicels 8–66 (av 26) μm (FIGS. 14–29). Zoospores produced after stimulation of low temperature (5 C, 10 min) and light. Sexual stage was homothallic, abundantly producing oogonia in culture media B-CA, B-LBA, frequently with tapered bases and 21.6–39.6 (av 30.4) μm diam. Antheridia were amphygynous and small at 7.2–14.4 × 6–12 (av 11.2 × 8.5) μm. Oospores were plerotic, 21–38.9 (av 30.3) μm diam with wall thickness 2.4–3.6 (av 3.2) μm (FIGS. 35–42). Immature oospores showed the presence of thick walls (FIGS. 30–35) and mature oospores had thinner walls at 2.4–3.6 (av 3.2) μm (FIGS. 36–45). Large ooplasts in mature oospores were frequently present, 13.2–20.4 (av 3.2) μm diam. (FIGS. 36–45).

Holotype. UNITED STATES OF AMERICA, Hawaii, Island of Hawaii, from *Morinda citrifolia* var. *citrifolia* (Indian mulberry, noni), Rubiaceae (Coffee family) infected with black flag disease of noni in the Puna District (Noni Farm Road), 10 Oct 2005, collector Scot C. Nelson. Isolate Ph697.P238. BPI 878721 (Dried culture on baby carrot agar). *Ex-type:* CBS 121982.

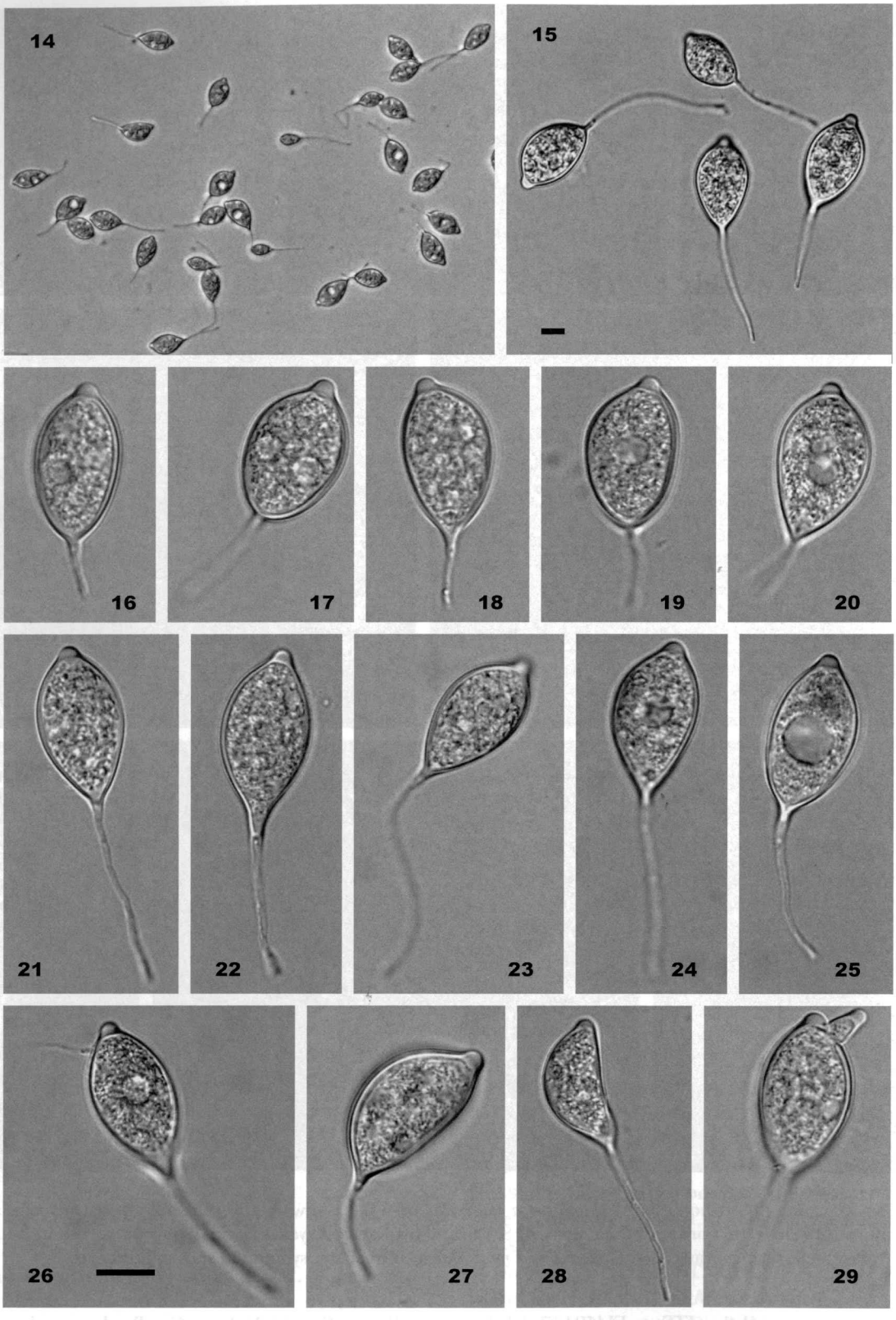


FIGS. 6–13. 6. Colony morphology of *Phytophthora morindae* on PDA, showing the moderate aerial mycelia and a fine chrysanthemum pattern at the border of the colony. 7, 8. Curling hyphae of *Phytophthora morindae* observed in B-LBA culture media. 9–13. Production of sporangia of *Phytophthora morindae* in umbellate, sympodial sporangiophores.

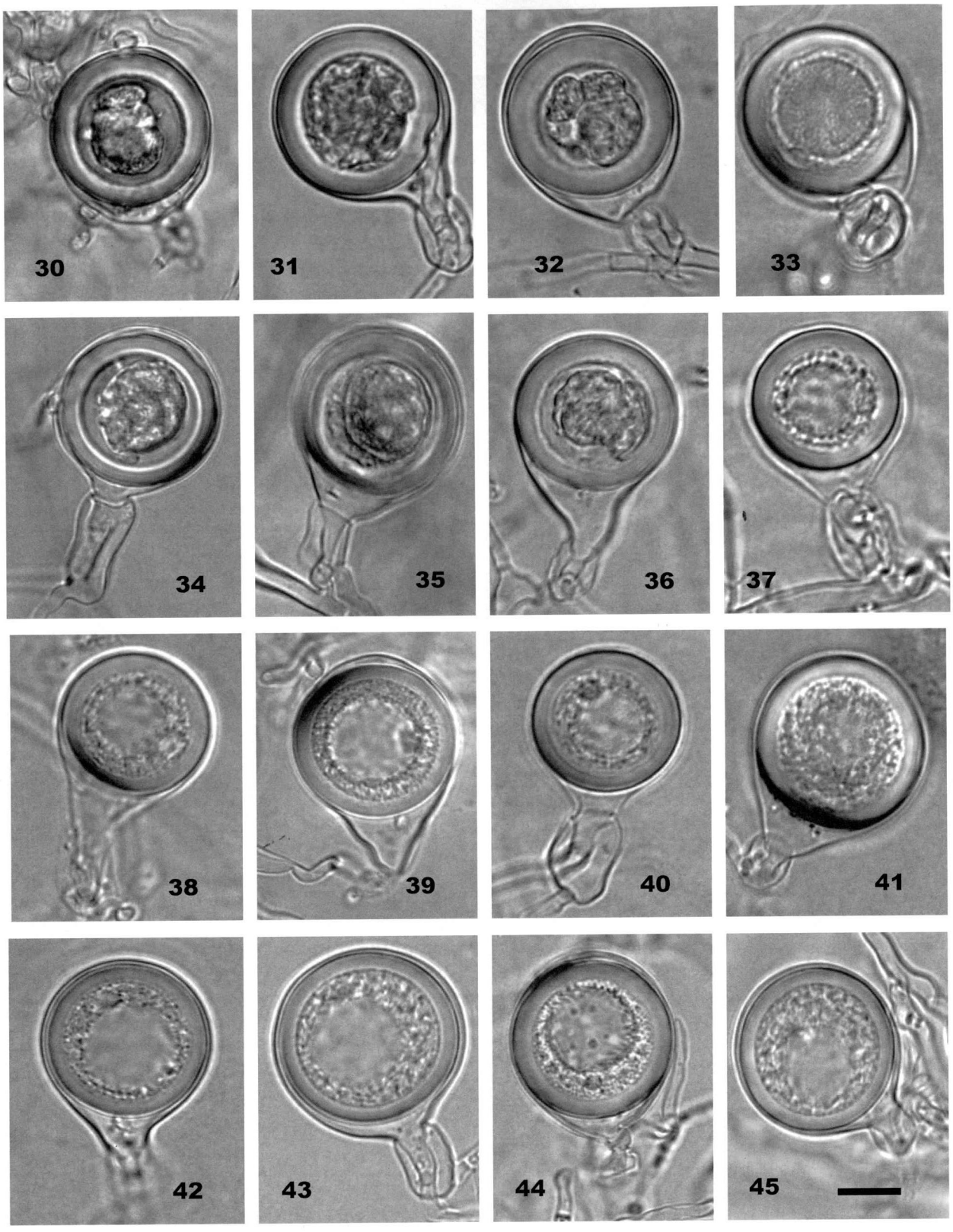
GenBank accession numbers: ITS = FJ469147, EF-1α = FJ469148.

Additional isolates. Isolate Ph698.P07.01, same host and same disease in the Puna District (Highway 137 near intersection of Kamaili Road), 3 Oct 2007, collector Scot C. Nelson. GenBank accession numbers: ITS = GQ166763, EF-1α = GQ166767.

Isolate Ph699.P07.02, in the Puna District (forest adjacent to Highway 137, between Kamaili and Pohoiki roads), 3 Oct 2007, collector Scot C. Nelson.



FIGS. 14–29. *Phytophthora morindae* asexual stage. Typical shapes of sporangia showing the presence of caducous medium to long pedicels. Bars = 10 μm.



FIGS. 30–45. *Phytophthora morindae* sexual stage. Immature oospores show the presence of thick walls (30–35). Mature oospores with thinner walls (36–45). Oogonia with tapered bases (35–42). Bars = 10 μm.

GenBank accession numbers: ITS = GQ166764 , EF-1α = GQ166768.

Isolate Ph700.P07.04, same host and same disease in the Puna District (forest adjacent to Highway 137, between Kamaili and Pohoiki roads), 3 Oct 2007, collector Scot C. Nelson. GenBank accession numbers: ITS = GQ166765, EF-1α = GQ166769.

Isolate Ph701.P07.04, in the Puna District (adjacent to Highway 137, near MacKenzie State Park entrance), 3 Oct 2007, collector Scot C. Nelson. GenBank accession #s: ITS = GQ166766, EF-1α = GQ166770.

Phylogeny.—Sequence analysis of the internal transcribed spacer rDNA (FIG. 46) and translation elongation factor 1 alpha (FIG. 47) demonstrated that *P. morindae* is in clade 10 and closely related to but distinct from *P. kernoviae.* The two species differ by 39 bp in the ITS and 36 bp in the EF-1α. *P. morindae* and *P. kernoviae* also are closely related to species in ITS clade 10, including *P. boehmeriae* and the recently described *P. gallica* (FIG. 46), than to other members in main cluster of *Phytophthora* (data not shown).

Pathogenicity tests.—Trials with noni plants having inoculated foliage within either clear or opaque (black) plastic bags up to 7 d after inoculation were not conducive for reproducing symptoms. Rectangular plugs of pathogen colonies grown on CMA also did not reproduce symptoms. Only when plants inoculated with aqueous suspensions of sporangia were placed under mist irrigation (10 s every 15 min) were symptoms readily produced. Typically symptoms typical of black flag disease appeared at approximately 7–10 d after inoculation. Blight rapidly developed within 2 wk of inoculation, with lesions expanding at approximately 1 cm diam or greater every 7 d.

Symptoms were reproduced most consistently where aqueous suspensions of sporangia were placed at the receptacle-like interface between stems and stipules at ambient temperature on a misting bench in Hilo, Hawaii, in 60% shade. Misting was 10–15 s at 15 min intervals. Diurnal temperatures in the shade house averaged approximately 21–26 C. Pathogenicity was confirmed by using sporangia as inoculum on plants held under the misting regime described above. Symptoms did not develop on control plants.

Major symptoms that were associated with the black flag disease on naturally infected plants were reproduced on the inoculated plants, including black veins, leaf blight, black flags and fruit rot. Rapid infection of stem stipules was common. Stipules showed dark, water-soaked lesions adjacent to the stem 4–7 d after inoculation. Symptoms spread from stipules into and along stems, where thin black streaks developed, and into leaf petioles. Entire stems and petioles eventually were colonized and turned dark black. Petioles often collapsed within 10 d after inoculation. In some cases leaf symptoms extended well beyond veins, turning them and the surrounding tissue black. Older, lower stipules and noni leaves usually were the first to show symptoms.

In some trials detached green fruits and their young flowers were inoculated with aqueous suspensions of sporangia and enclosed in moisture chambers at approximately 24 C. Within a few days fruits showed dark, water-soaked lesions and flowers were blighted. Abundant visible sporulation of the *P. morindae* on the surface of host tissues occurred during incubation of diseased plants in the moisture-saturated air.

After symptoms developed tissues were observed microscopically and plated out and the pathogen cultured on water agar as described. Sporangia and oospores of the organism typically could be observed on the diseased, necrotic tissues, and isolations yielded colonies that were morphologically identical to those used as sources of inocula.

To confirm the presence of *P. morindae* in infected tissues of diseased plants the margins of advancing lesions of symptomatic tissues were sectioned by hand, stained with cotton blue and observed microscopically at 100×. Embedded oospores and sporangia were observed within symptomatic tissues. The mycelium was consistently observed within the cortex and phloem of stems, petioles and leaves, where it was associated with the dark necrosis. The pathogen rapidly colonized the tissues of *M. citrifolia* var. *citrifolia,* leading to blackened veins resembling symptoms of some diseases caused by plant-pathogenic bacteria.

To date this is the only variety of noni tested for susceptibility. The two other recognized varieties of noni, *M. citrifolia* var. *bracteata* and *M. citrifolia* Potteri (a green and white variegated form) have yet to be evaluated as hosts for the new pathogen. Symptoms are not present in the field on these two varieties, but neither is known to grow within the confined area where the disease epidemics occur, and they are not farmed as crops in Hawaii but instead exist as ornamental plants in landscapes or as specimens in botanical gardens.

DISCUSSION

A previously unknown *Phytophthora* sp. associated with a severe foliar blight and fruit rot disease of noni (Indian mulberry, *Morinda citrifolia* var. *citrifolia*) was identified in this study. A number of unique morphological characteristics and the phylogenetic analyses of the DNA sequence data for the internal

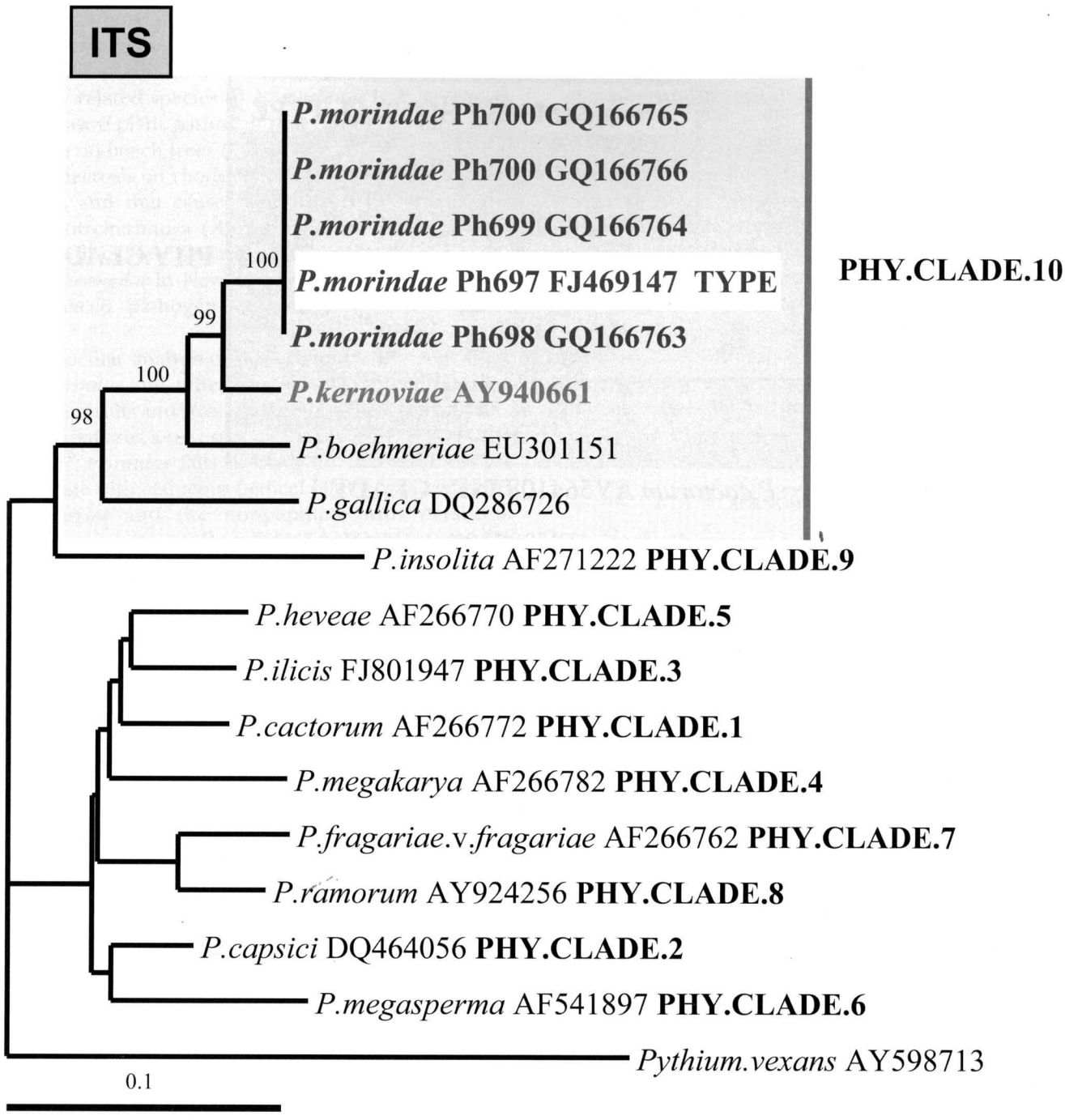


FIG. 46. Neighbor joining phylogenetic tree based on the ITS rDNA region of *Phytophthora* clade 10, showing the position of *Phytophthora morindae* in relation to other known taxa in the clade. Selected sequences from other clades are included. *Pythium vexans* is outgroup. Bar = number of nucleotide substitutions per site.

transcribed spacer rDNA (ITS) and translation elongation factor 1 alpha (EF-1α) regions of this taxon support the concept that this organism was a previously undescribed species, and we describe it here as *Phytophthora morindae* sp. nov. *Phytophthora morindae* is a self-fertile (homothallic) species with ellipsoid, ellipsoid with tapered bases, obpyriform sporangia, which are papillate and produced in umbellate, sympodial sporangiophores. Additional characters for the sporangia are their caducous habit and medium to long pedicels. The morphological characters of the asexual stage of *P. morindae,* including sporangia and sporangiophores, are similar to those observed in *P. tropicalis.* However the sexual

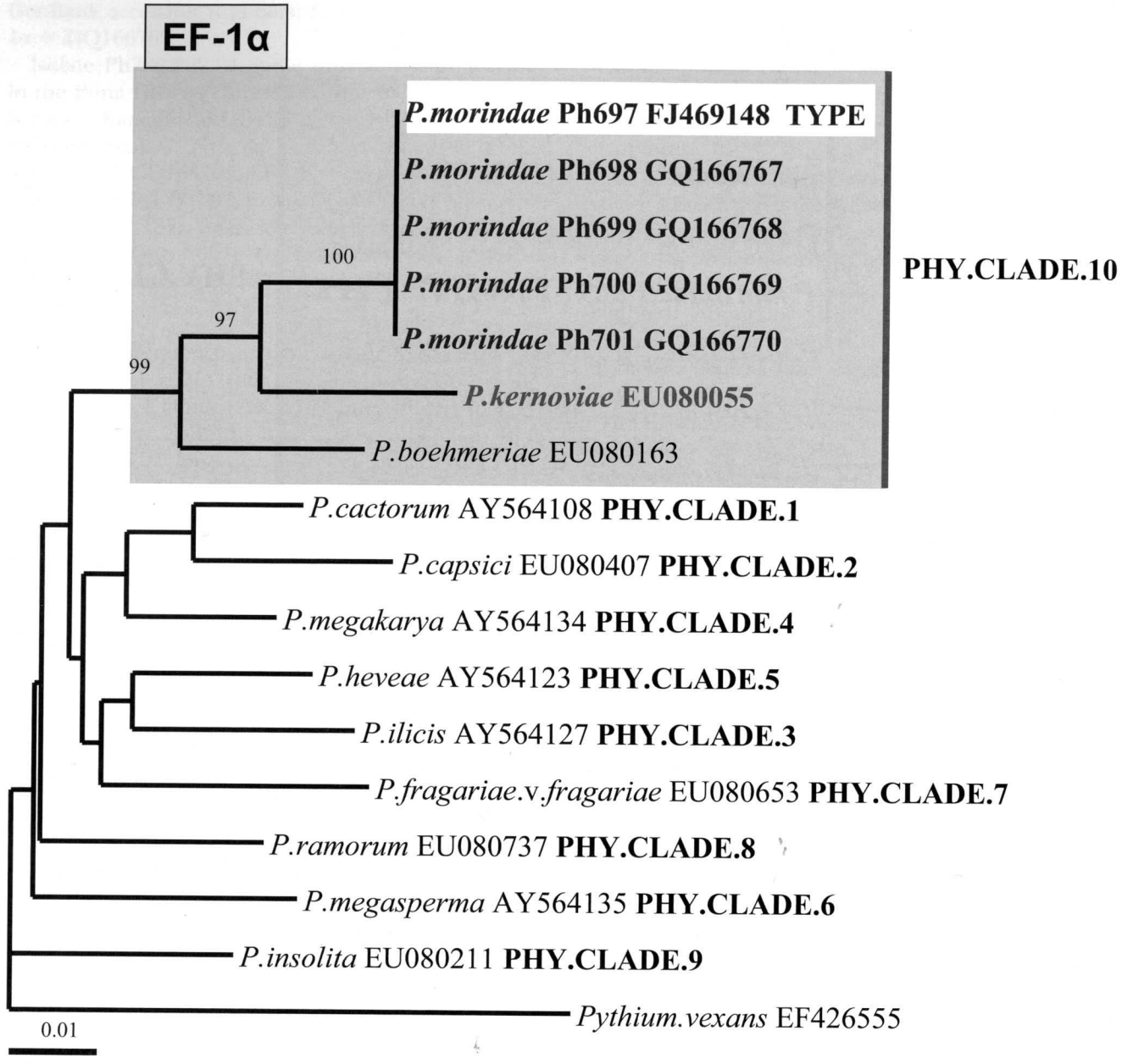


FIG. 47. Neighbor joining phylogenetic tree based on the translation elongation factor 1 alpha (EF-1 α) region of *Phytophthora* clade 10 showing the position of *Phytophthora morindae* in relation to other known taxa in the clade. Selected sequences from other clades are included. *Pythium vexans* is outgroup. Bar = number of nucleotide substitutions per site.

phases of both species are different, including the homothallic character of *P. morindae* and the heterothallic character of *P. tropicalis*. *Phytophthora morindae* also is characterized by the production of oogonia with tapered bases, thick oospores walls and amphigynous small antheridia, while *P. tropicalis* is characterized by the production of oogonia with nontapered bases and bigger amphigynous antheridia (13 × 15 μm). Morphological comparison of the isolates of this *Phytophthora* and those published for the valid 95 reported taxa shows that *P. morindae* is a distinct species. Molecular analysis of sequences of the internal transcribed spacer rDNA region (ITS) of this taxon and those from other *Phytophthora* species from GenBank and the *Phytophthora* database indicates that the new species is most closely related to species in clade 10, including *P. kernoviae*, *P. boehmeriae* and the recently described *P. gallica*. Molecular analysis of sequences of the translation elongation factor 1 α (EF-1α) of this taxon and those from other *Phytophthora* species from GenBank and the *Phytophthora* Database indicates that the new

species is most closely related to *P. kernoviae* and *P. boehmeriae*. No sequences of the EF-1α of *P. gallica* are available at this time in either database. The most closely related species to *P. morindae* is *P. kernoviae*, an invasive plant pathogen that causes bleeding stem lesions on beech trees (*Fagus sylvatica*) and foliar and stem necrosis on rhododendron, pieris and magnolia in UK and that causes necrosis on the shoots and fruits of cherimoya (*Annona cherimola*) and isolated from soil in New Zealand (Webber 2007). The status of *P. kernoviae* in New Zealand, either as a native or introduced pathogen, remains unknown (Webber 2007).

Molecular analysis of the ITS and EF-1α regions of *P. morindae* and other species validate the morphological results and strongly supports the description of *P. morindae* as a new species. In the phylogenetic ITS tree *P. morindae* falls in clade 10 that contains the papillate with caducous pedicel of *P. kernoviae* and *P. boehmeriae* and the nonpapillate with persistent sporangiophore of *P. gallica*. Sequences of the ITS rDNA region of *P. morindae* differ from *P. kernoviae* by 39 base pairs and 36 bp positions in the EF-1α region. The main morphological differences between these two species are the production of sympodial sporangiophores in *P. kernoviae*, while in *P. morindae* sporangiophores are umbrella shape. Although *P. kernoviae* and *P. morindae* are similar in the production of sporangia with caducous medium size pedicels, they differ in the presence of sporangia with long pedicels observed only in *P. morindae*. Both species are similar in sexual phase characteristics, including that both are homothallic and produce similar smooth oogonia with tapered bases, amphigynous small antheridia and thick-walled oospores. The main difference in the sexual stage between the two species appears to be in the presence of clear ooplasts and the presence of long and thin antheridia in *P. morindae*. In addition neither species produces chlamydospores or hyphal swellings. Another close relative of *P. morindae* based on molecular data of the ITS rDNA and EF-1α is *P. boehmeriae*. This species has been recorded on hosts in China, Australia and Greece (Erwin and Ribeiro 1996, Gallegly and Hong 2008). *Phytophthora morindae* and *P. kernoviae* differ morphologically from *P. boehmeriae* in their large sporangial pedicels compared with those of *P. boehmeriae* that are short, up to approximately 5 μm. *Phytophthora morindae*, *P. kernoviae* and *P. boehmeriae* are homothallic with caducous sporangia and adapted to aerial living, while the other species in clade 10 recently described as *P. gallica* is sterile and morphologically and ecologically more similar to several nonpapillate species of ITS clade 6 (Jung and Netwatal 2008). *Phytophthora morindae* can be distinguished from other species with caducous pedicels, including *P. meadii*, *P. botryosa* (Erwin and Ribiero 2006) and *P. nemorosa* (Hansen et al 2003), by its characteristic ellipsoid sporangia having tapered bases.

Phytophthora morindae has been found in a localized area in the Puna District near Opihikao on the island of Hawaii, and at present the origin, geographical distribution, infection and dispersal models are unknown and require further investigation. Additional research is required to determine the host range and the genetic variability of the species. The fact that this organism at the present is the closest relative of *P. kernoviae*, an important invasive pathogen, makes *P. morindae* an interesting species in *Phytopththora* studies, especially because *P. kernoviae* is not known to exist in Hawaii.

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